CHAPTER 8

8 RESULTS AND DISCUSSION

Section-A

TRIAZOLE

8.1 Synthesis of 3-(N-substitutedcarboxamidoalkylthio)-4(H)-1,2,4-triazoles, their corresponding sulfoxides and sulfones

(Scheme 1 & Scheme 2)

8.1.1 Chemistry

3-Mercapto-(4H)-1,2,4-triazole (2) was synthesized by cyclization of 1-formyl thiosemicarbazide (1) as the essential pharmacophore. Various N-substituted chloroacetamides (5) and N-substituted β-chloropropionamides (10) were synthesized by an appropriate method given in the literature, by condensing different primary aromatic amines (4) with chloroacetyl chloride (3) and β-chloropropionylchloride (9), respectively as an acylating agent. These compounds were characterized by TLC, melting point and IR spectra that showed characteristic absorption bands at 3250 and 1650 cm⁻¹ of N-H and C=O respectively. These different N-substituted chloroacetamides (5) and N-substituted β-chloropropionamides (10) were condensed with 3-mercapto-(4H)-1,2,4, triazole (2) under Schotten-Baumann reaction condition by conventional as well as microwave irradiation method to obtain various 3-(N-substituted carboxamidoalkylthio)-(4H)-1,2,4-triazole derivatives (6a-k) and
(11a-k) as shown in Scheme 1 and Scheme 2. The infrared spectra of 3-mercapto-(4H)-1,2,4-triazole (2) showed characteristic absorption band at 2585 cm\(^{-1}\) was attributed to SH, which was disappeared by the formation of 3-(N-substituted carboxamidoalkythio)-(4H)-1,2,4-triazoles (6 and 11). Similarly the \(^1\)H NMR spectra of the synthesized triazole (2) showed one characteristic signal at δ13.8-13.95, which was absent in the \(^1\)H NMR spectra of substituted triazoles (6 and 11). The absence of these absorptions due to SH established that the triazoles had converted to 3-(N-substituted carboxamidoalkythio)-(4H)-1,2,4-triazoles by reacting with N-substituted chloroacetamides and N-substituted β-chloropropionamides. In IR spectra of compound (6 and 11) showed a peak at 3570 to 3221, 3105 to 3037, 1695 to 1616, 1600 to 1491, 1247-1110 due to Ar-NH, Ar-CH, CO, 2\(^\text{nd}\)NH, and C=N, respectively. In the \(^1\)H NMR spectrum of compound (6), additional signals derived from –CH\(_2\), NH and aromatic protons were observed at 2.6-5.5, 8.2-11.1 and 6.8-7.7 ppm respectively, while for compound (11), additional signals derived from -COCH\(_2\), -SCH\(_2\), NH and aromatic protons were observed at 2.2-2.9, 3.7-3.9, 9.4-10.2 and 7.0-7.8 ppm respectively. Further evidence for the structure assigned to 6 and 11 was obtained by recording mass spectra and elemental analysis. The mass spectrum of 6 and 11 showed a molecular ion peak which corresponding to its molecular weight. Other peaks appearing were in accordance with the fragmentation pattern.
Elemental analysis supported the structure of various synthesized compounds (6 and 11).

In view of the utility of microwave irradiation and the biological importance of 3-(N-substituted carboximidoalkylthio)-(4H)-1,2,4-triazoles, it was thought worthwhile to develop a convenient microwave method for the synthesis of the title compounds. As indicated by TLC analysis, a maximum of 6 minutes of heating suffices to produce nearly complete conversion. However, in absence of microwave heating, poor conversions to triazole derivatives were realized, when the reactions were stirred at 60-80 °C for 36 h. This method appeared to be rapid and economical with a wide range of applications.

Moreover, we had synthesized various sulfoxides and sulfones selectively from the corresponding sulfides with great purity, high yields and environmentally friendly way. This was achieved with good success by the described method. Much of the current work in the area of synthesis of sulfoxides and sulfones from sulfides focuses on the use of transition metal catalyzed processes. However, a large number of such oxidation reactions often require the use of toxic metal reagents or catalysts. Consequently, from a green chemistry standpoint, it is very important to develop a “green” oxidation system for chemical synthesis. Oxone® was proved as an ideal “green” oxidant due to its strength and lack of toxic by-products.
The traditional reagents used in oxidation of sulfides to sulfoxides gave mixture of the corresponding sulfoxides and sulfones and also operating conditions are quiet difficult. These problems associated with the mostly used oxidants were successfully overcome by using this simple, effective and efficient solid-state oxidation method employing oxone®.

The said “green” synthetic method for the solid-state synthesis of sulfoxides and sulfones from sulfides using oxone® gave high and excellent yields of the products and thus proved extremely useful.

The reaction involves a transfer of oxygen radicals towards the sulfide through the formation of peroxo radicals due to the presence of catalyst. It was initially observed that oxone® alone did not initiate the reaction but the peroxo radicals formed causes the reaction to proceed in the desired direction.

The formation of corresponding sulfoxides (7 from 6 and 12 from 11) and sulfones (8 from 6 and 13 from 11) were substantiated by spectral studies. IR spectrum of the compound (7 and 12) exhibited NH stretching at 3572-3226 cm⁻¹ and SO band in the region 1135-1170 cm⁻¹. In the ¹H NMR, appearance of a downfield shift at –SCH₂ and NH proton signals for sulfoxides (7) and –SCH₂, -COCH₂ and NH proton signals for sulfoxides (12) when compared with the sulfides (6) and (11) indicated that oxidation reaction occurred. IR spectrum of compound (8 and 13) exhibited NH stretching at 3578-
3230 cm\(^{-1}\) and two series of SO\(_2\) stretching bands, one between 1311 and 1367 cm\(^{-1}\) and the other one between 1120 and 1152 cm\(^{-1}\) were seen. In the \(^1\)H NMR, appearance of a downfield shift at \(-\text{SCH}_2\) and NH proton signals for sulfone compounds (8) and \(-\text{SCH}_2\), -COCH\(_2\) and NH proton signals for sulfone compounds (13), when compared with the sulfides (6) and (11) and sulfoxides (7) and (12), indicated that oxidation reaction occurred. But it was hard to say that isolated product has either one or two oxygen in its molecule. Additional evidence was provided by mass spectra and elemental analysis of the compounds. In the mass spectra of the compounds (7 and 12) and (8 and 13), molecular ion peaks were observed for all compounds at different intensity verifying the molecular weight of a sulfoxide and sulfone compound.

8.1.2 Biological results

8.1.2.1 Analgesic activity

All the compounds 6a-k and 11a-k were tested for their analgesic activity by using Eddy’s hot plate technique and exhibited percent inhibition in the range of 0%-413% and 0.3%-303%, respectively.

Scheme 1 The compound 6e and 6g have shown highly potent analgesic activity compared to standard drug Tramadol (TMD) while
the others were also having the analgesic effect but was not significant. The compound 6h and 6i didn’t have any effect.

**Scheme 2** The compound 11a has shown highly potent analgesic activity compared to standard drug, whereas rest of the tested compounds did not show significant analgesic activity in the model used.

**8.1.2.2 Anti-inflammatory activity**

The synthesized compounds (6a-k and 11a-k) were tested for their anti-inflammatory activity using carrageenan induced rat hind paw edema method of Winter et al. Data of anti-inflammatory activity was expressed as mean ± SEM, and the student’s t-test was applied to determine the significance of the difference between the control group and rats treated with the test compounds.

**Scheme 1** The synthesized compounds have shown significant anti-inflammatory activity compared to standard drug. The compound 6h had shown most significant activity i.e. 47.84% inhibition as compared to standard drug Diclofenac sodium having 23.14% inhibition while others also have considerable activity. Some compounds like 6e, 6j were found with no significant activity.

**Scheme 2** The compound 11a had shown equipotent activity compared to standard drug, whereas the incorporation of chloro, nitro, methyl and methoxy groups into phenyl ring and replacement of
phenyl nucleus by benzyl enhanced the anti-inflammatory activity considerably. Among these triazole derivatives 11b and 11d showed the maximum anti-inflammatory activity. It was also observed that the substitution at para position is more potent than the ortho and meta positions. It was interesting to note that the compound 11a manifested both anti-inflammatory and analgesic activities.

8.1.2.3 Molecular docking study

In order to investigate mechanism involved in anti-inflammatory activity, we have carried out Molecular docking of compound 11a which had shown comparable inhibition as that of standard drug Diclofenac Sodium. Before going for docking studies, we have focused on stereochemical aspects of compound under study.

Tautomerism by definition concerns all molecules which can readily interconvert into isomers by transfer of a chemical group. Tautomerism being very complex is related to several phenomena: different types of migrating groups such as electrofuge or a nucleofuge, cationotropic/anionotropic properties, valence tautomerism, zwitterionic tautomerism, tautomerism related to migration of neutral groups in molecules and migration of bonds or ring-chain tautomerism.\textsuperscript{1-3}

Sadowski et al.\textsuperscript{4} have addressed the issue of tautomery together with protonated molecules using a tautomer and protonation
preprocessor for virtual screening. Subsequently, the top scored compounds and tautomers were compared to each other and revealed new characteristics of the ligand binding. It can be postulated that when active site favors one particular tautomeric form over the other, this could reveal new lead structures and may be omitted in a classical, tautomerism-disregarded screening. Moreover, finding tautomeric hits may initiate new case studies of tautomery-dependent ligand–protein interaction.

Stereochemistry of compound 11a revealed characteristic ring chain and amide-amidic tautomerism. In order to investigate which tautomeric form of compound 11a predominantly involved in COX-1 inhibitory mechanism, its selectivity and stable interaction with COX-1 receptor, we have derived various tautomeric forms of compound 11a and carried out docking study of six principal tautomeric forms of compound 11a with COX-1. Figure 8.1 showing six principal neutral possible tautomeric forms for compound 11a.
Figure 8.1: Six principal neutral possible tautomeric forms for compound 11a.

Docking algorithms

Docking programmes “direct” and “unbiased” have their own advantages and disadvantages. Direct docking software such as DOCK has the benefit of speed but it has disadvantage of making assumptions about the potential energy landscape to save computational time. Unbiased methods such as Auto Dock, FTDOCK and MOE-Dock have few assumptions about the potential energy
landscape. Thus at the expense of computation time, they find final docked solutions that the direct method might have missed. Here we report the use of MOE-Dock by Chemical Computing Group Inc.\(^6\), which has the advantage of flexible docking, integration with a graphical interface as well as with other modules such as analysis, molecular mechanics, and molecular dynamics.

**Docking simulations**

In MOE, London dG scoring is used as default setting to calculate the exact confirmation and configuration of the ligand for finding the best molecule with minimum binding energy and to develop potential drug molecules against the disease. The London dG scoring function estimates the free energy \(\Delta G\) of binding of the ligand from a given pose. The functional form is a sum of terms:

\[
\Delta G = c + E_{\text{flex}} + \sum_{h\text{-bonds}} C_{HB} f_{HB} + \sum_{m\text{-lig}} C_{M} f_{M} + \sum \Delta D_i
\]

Where \(C\) represents the average gain/loss of rotational and translational entropy; \(E_{\text{flex}}\) is the energy due to the loss of flexibility of the ligand (calculated from ligand topology only); \(f_{HB}\) measures geometric imperfections of hydrogen bonds and takes a value in \([0,1]\); \(C_{HB}\) is the energy of an ideal hydrogen bond; \(f_{M}\) measures geometric imperfections of metal ligations and takes a value in \([0,1]\); \(C_{M}\) is the
energy of an ideal metal ligation; and $D_i$ is the desolvation energy of atom $i$. The difference in desolvation energies is calculated according to the formula,

$$\Delta D_i = c_i R_i^3 \left\{ \iiint_{A,B} |u|^{-6} \, du - \iiint_{A,B} |u|^{-6} \, du \right\}$$

Where $A$ and $B$ are the protein and/or ligand volumes with atom $i$ belonging to volume $B$; $R_i$ is the solvation radius of atom $i$ (taken as the OPLS-AA Van der Waals sigma parameter plus 0.5 Angstrom); and $C_i$ is the desolvation coefficient of atom $i$. Atoms are categorized into ~12 atom types for the assignment of the $C_i$ coefficients. MOE 2009.10 was run on a Windows XP based Pentium IV 2.66 GHz PC (with 2GB RAM).\textsuperscript{7}

**Substrate docking**

Structure of all the tautomers were derived in MOE programme by using Protomer option. Before molecular docking; the 3D structure of ligand was optimized. The pdb 3N8X\textsuperscript{7} (1) was subjected to energy and residue optimization by protonate 3D option in MOE programme.
The health of protein was ascertained by Ramchandran plot (Figure 8.2).

**Figure 8.2:** Ramchandran Plot for pdb 3N8X (1) after energy and residue optimization

Substrates were docked within the active site using the Monte Carlo docking procedure of MOE and repeated cycles of protein and substrate minimization. During the initial stage of the docking procedure, the side chains of the protein were fixed. The best-ranking docking modes of the ligands were identified and energy minimized in the protein while allowing full side chain flexibility. Energy of conformation of different tautomeric forms of compound 11a is given in Table 8.1.
Table 8.1 Energy of conformation of different tautomers of 11a

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Where \( E_{\text{con, T}} \) is the energy of conformation of tautomers 1, 2, 3, 4, 5 & 6.
Results and discussion

All six tautomers (Figure 8.1) were used as ligands in the binding interaction studies with the active site of the COX-1 receptor. Docking of these tautomers to the active site revealed that tautomer 3 and 5 have zero docked energy of conformation while tautomer 2 have the lowest docked energy of conformation, followed by tautomer 1, 6 and 4 (Table 8.1). These results are parallel to the highest % inhibition observed experimentally for compound 11a. The lowest conformational energy tautomer 2 may be the best conformation for compound 11a to interact with COX-1 receptor.

All the ligands showed reasonably low docked energy of conformation indicating that the docked conformers were in their most favourable conformations. The spatial arrangement of the six tautomers bound to the active site of COX-1 is shown in Figure 8.3.
Figure 8.3: Connolly surface representations of the active site of Cyclooxygenase-1 with the bound tautomers $a(T1)$, $a(T2)$, $a(T3)$, $a(T4)$, $a(T5)$ and $a(T6)$, shown in Ball and Stick model. Connolly surface of the active site of the Cyclooxygenase-1 is coloured according to a charge spectrum: Blue colour for H-bonding region, white colour
indicates lipophilic area while red colour is used to reveal mild polar area.

All the tautomers have shown characteristic interaction with COX-1 receptor as indicated by their docked energy of conformation and their hydrogen bonding pattern.

**Interactions between tautomers and residues in cyclooxygenase-1**

Hydrogen bond analysis was performed on the docked COX-1 and different tautomer complex to determine the possibility of hydrogen bonding or salt bridge formation between various tautomers and the active site of COX-1. The criteria for hydrogen bond interaction used, when the distance between the hydrogen and the heteroatom was within the range of 2.5-3.5 Å and the bond angle was at 109°-110°. Overall, these ligands exhibited binding interactions within the active site and the residues as suggested by Bazan and Fletterick (Bazan and Fletterick, 1989).

Interestingly, in our study, all the ligands were observed to form hydrogen bond with COX-1 active site residue except tautomer 2 which is involved in strong hydrophobic interaction with COX-1 as indicated in Figure 8.4.

It was observed that among the various tautomers, tautomer 1 interacted with residue, Arg (A) 120 through arene–cation interaction and forms hydrogen bond with Glu (A) 524. Percent of H-bonding was found to be 81% (Figure 8.4). Nitrogen atom of triazole nucleus acts...
as hydrogen bond donor and formed a hydrogen bond with Glu (A) 524. Tautomer 2 was not involved in hydrogen bonding but it interacted through hydrophobic and polar interaction with the residue Ser (A) 353, Met 522, Ile 523, Leu 384, Phe 518, Leu 384, Gly 526, Tyr 385, Ala 527, Trp 387 and Ser 530. In tautomer 3, protonated nitrogen atom of triazole nucleus acts as hydrogen bond donor and was involved in H-bond interaction with residue Glu (A) 524, whereas triazole ring interacts with Arg (A) 120 through arene-cation interaction. Tautomer 4 has shown interesting H-bond interaction with residues Arg (A) 120, Glu (A) 524 and His 513. In this interaction, hydroxyl group formed by tautomerism was reasonably involved in hydrogen bonding interaction whereas nitrogen atom of triazole nucleus acts as hydrogen bond donor and form hydrogen bond with His 513. Hydroxyl group has shown strong hydrogen bonding with Arg (A) 120 in which percent of hydrogen bonding was found to be 64% with optimum interatomic distance of 1.6 Å, whereas Glu (A) 524 showed next strong H-bond interaction with the percent of H-bonding was 52% having interatomic distance of 2.4 Å while nitrogen revealed about 19 % H-bond interaction with residue His 513 with interatomic distance of 3.0 Å. Again in Tautomer 5, hydroxyl group formed by tautomerism was involved in H-bond interaction with the residue
Glu 524 wherein hydroxyl group acts as hydrogen bond donor while triazole nucleus showed arene-cation interaction with the residue Arg 120. In this interaction percent of hydrogen bonding was found to be 70% with optimum interatomic distance of 2.8 Å between Glu 524 and hydroxyl group formed by tautomerism.
Interaction of Tautomer-2

H-Bond interaction of Tautomer-1

H-Bond interaction of Tautomer-4
H-Bond interaction of Tautomer 5

H-Bond interaction of Tautomer 6

**Figure 8.4:** Showing hydrogen bonding interaction of tautomer-1, 2, 3, 4, 5 and 6.

In case of tautomer 6, benzene nucleus was involved in arene-cation interaction with Arg 120 residue while protonated nitrogen of triazole acts as hydrogen bond donor and form hydrogen bond with proline 86 residue of COX-1.

In order to get useful results that may be valuable in future drug designing, we have carried out receptor based electrostatic analysis also.
Electrostatic analysis of Tautomer 1

Electrostatic analysis of Tautomer 2

Electrostatic analysis of Tautomer 3

Electrostatic analysis of Tautomer 4

Tautomer 5

Tautomer 6
Figure 8.5: Receptor based electrostatics regions in the active site of Cycloxygenase-1. Blue = donor, Red = acceptor and white = hydrophobic regions.

Receptor based electrostatic regions around various tautomers are shown in Figure 8.5. In tautomer 1, blue region around triazole nucleus reveals the hydrogen bond donor region and slight white region near triazole suggest the requirement for any hydrophobic substituent that may enhance the activity whereas sulfur bridge don’t have any contribution in enhancing the hydrophobicity of tautomer-1. Further aromatic substituents near amide linkage do not have any participation in hydrophobicity but substitution of any hydrogen bond donor may enhance activity of tautomer which is shown by blue region around aromatic ring.

Receptor based electrostatic regions in tautomer 2 reveals some interesting facts. Blue region around triazole nucleus had shown a requirement for any hydrogen bond donor as well as hydrogen bond acceptor which may enhance the interaction between COX-1 and tautomer 2. Compound 11a in 2nd tautomeric did not show any hydrogen bonding interaction it might interact with COX-1 via hydrophobic interaction. Receptor based electrostatic regions reveals strong hydrophobic nature of aromatic ring which is represented by white surface around aromatic ring. Further blue region around aromatic ring suggest the requirement of any hydrogen bond donor as
well as acceptor for increasing the activity of compound 11a. Carbon chain which is connecting the sulfur atom and amide linkage may enhance the hydrophobicity of tautomer 2 of compound 11a.

In tautomer 3 of compound 11a, receptor based electrostatic regions showed blue region around the protonated nitrogen of triazole nucleus reveals hydrogen bond donor nature of nitrogen and suggest the substitution of any H-bond donor may enhance the activity. Carbon atom in triazole ring may impart hydrophobicity to the tautomer 3 of compound 11a.

In tautomer 4 of compound 11a, aromatic ring near amide link shows strong hydrophobic region indicated by white area around aromatic ring which may involved in hydrophobic interaction with COX-1. Further, red and blue region around aromatic ring suggests the substitution of any hydrogen bond donor as well as hydrogen bond acceptor may enhance the affinity of compound 11a towards COX-1. Fourth tautomeric form of compound 11a gave rise to formation of one hydroxyl group near aromatic ring that form hydrogen bonding interaction with Cyclooxygenase residue, indicated by blue and red region around hydroxyl group. Presence of both blue and red region around hydroxyl group may suggest the dual nature, wherein oxygen atom acts as hydrogen bond acceptor and proton in hydroxyl group acts as hydrogen bond donor. Blue region around
triazole ring reveals the hydrogen bonding nature of triazole nitrogen with slight hydrophobicity enhanced by carbon atom in triazole ring.

Receptor based electrostatic analysis of tautomer 5 reveals characteristic blue region covering the triazole nucleus of compound 11a suggest that substitution of any hydrogen bond acceptor group may enhance the affinity of ligand towards receptor. Presence of sulfur bridge may enhance hydrophobic interaction with receptor. Hydroxyl group formed by tautomerism imparting donor feature (shown by blue colour, Figure 8.5) to the compound 11a, help in enhancing the affinity towards receptor. Further, presence of red colour contour around aromatic ring suggests that the substitution of any hydrogen bond acceptor enhances the hydrogen bond interaction as well as affinity towards receptor.

Electrostatic analysis of tautomer 6 shows blue contour around triazole nucleus indicating the hydrogen bond donor nature of triazole nitrogen. Further sulfur bridge connecting triazole ring shows characteristic white region indicating sulfur may be involved in enhancing the affinity of compound 11a towards the hydrophobic region of COX-1 receptor.

**Selective inhibition of cox-1**
In crystallization experiments, it was reported that all COX-1 inhibitors interacted with the putative catalytic amino acid residue Tyr 385 and formed hydrogen bonds with Arg 120 and Tyr 355. In mapping, it was observed that 2-(4H-1,2,4-triazol-3-ylthio)-N-phenylacetamide 11a docked near the gate of COX-1. Among all six principle tautomeric forms of 2-(4H-1,2,4-triazol-3-ylthio)-N-phenylacetamide, tautomer 1 and 3 showed arene-cation interaction with Arg 120 and hydrogen bond interaction with Glu 524 residue of COX-1, whereas in tautomer 4, hydroxyl group (formed by tautomerism) form hydrogen bond with Arg 120 and Glu 524 residue of COX-1. Further tautomer 5 and 6 were interacted with COX-1 by arene-cation interaction and form hydrogen bond with glu 524 and pro 86.

Herein, different tautomeric forms of compound 2-(4H-1,2,4-triazol-3-ylthio)-N-phenylacetamide 11a interacted with COX-1 by hydrogen bonding indicates involvement of tautomerism in drug receptor interaction which may give rise to stable drug receptor complex. Further, binding of compound 2-(4H-1,2,4-triazol-3-ylthio)-N-phenylacetamide to Arg 120 and Glu 524 may block the gate of active site of COX-1 and interfere with the conversion of arachidonic acid to prostaglandin (PG) H2 in the active site of COX-1. It can be hypothesized that compound 2-(4H-1,2,4-triazol-3-ylthio)-N-phenylacetamide 11a binds with COX-1 in a selective manner wherein
tautomerism seems to be most important factor in selective inhibition of COX-1, particularly the hydroxyl group formed by tautomerism directly influence the selective inhibition of COX-1.

In the present study, interference in arachidonic acid binding channel might be possible mode of action of 2-(4H-1,2,4-triazol-3-ylthio)-N-phenylacetamide 11a, wherein tautomerism plays vital role in selective inhibition of COX-1.

8.1.2.4 Anxiolytic activity

Scheme 1 and Scheme 2

All the compounds (6a-k and 11a-k) were tested for their anxiolytic activity by using Elevated plus maze and it was observed that the control did not show any entry in open arm, and spent total time in enclosed arm. After the treatment with the standard drug Diazepam, there was significant increase in the time spent as well as percent number of entries in the open arm. After the treatment with triazole derivatives (6a-k and 11a-k), there was increase in percent time spent and number of entries in the open arm but the anxiolytic activity was found to be very less as compared to the standard drug.

8.1.2.5 Antimicrobial activity

The antifungal activity studies of the newly synthesized triazole derivatives 6a-k, 7a-k, 8a-k and 11a-k have been carried out against the fungi Candida albicans and Aspergillus niger and antibacterial activity have been carried out against four pathogenic organisms, viz.,
Staphylococcus aureus (G⁺), Klebsiella pneumoniae (G⁻), Escherichia coli (G⁻), and Pseudomonas aeruginosa (G⁻) whereas compounds 12a-k and 13a-k have been screened against Staphylococcus aureus (G⁺), Escherichia coli (G⁻), and Pseudomonas aeruginosa (G⁻). Norfloxacin and Ciprofloxacin were used as standard drug for antibacterial screening and Fluconazole was used as standard drug for antifungal screening.

Scheme 1 Among the compounds 6a-k, particularly, 6b, 6d and 6k having chloro substituent on phenyl ring have exhibited more activity at MIC value of 125 µg/ml against the bacteria, K. pneumoniae, E. coli, and P. aeruginosa but less activity against S. aureus at MIC value of 250 µg/ml as compared to other compounds. Moreover, corresponding sulfoxides and sulfones of these compounds (7b, 7d, 7k, 8b, 8d, 8k) were found to exhibit good activity at MIC value between 62.5 µg/ml and 125 µg/ml.

All compounds of the series have shown less activity against the tested fungi when compared to the standard with MIC values between 62.5 and >500 µg/ml. Particularly, compounds having nitro substituent (6c, 6e, 6h) on phenyl ring were more active than the other compounds in the series. It was also observed that sulfides were less active than their corresponding sulfoxides and sulfones, but no remarkable difference in activity between sulfoxides and sulfones was observed.
Scheme 2  Here, in case of 11a-k, same trend was observed as that of scheme-1. Compounds having chloro substituent (11b, 11d, 11k) on phenyl ring were more active as an antibacterial with MIC value of 125 µg/ml and compounds having nitro substituent (11c, 11e, 11h) were more active as an antifungal compared to other compounds of the series, with MIC value 125 µg/ml. There was no significant increase in antibacterial and antifungal activity with increase in carbon chain. Among the compounds 12a-k and 13a-k, compounds having chloro and nitro substituent exhibited remarkable antibacterial and antifungal activity with MIC values between 62.5 and 125 µg/ml. Particularly, among the compound tested, compound 12c, 12e, 12h, 13c, 13e and 13h were found more active as an antifungal than antibacterial.
8.2 Synthesis of 2-amino-6-[5-pyridine-4-yl-1,2,4-triazole-4[H]-phenyl-3-y]lthio)methyl]-4-arlynicotinonitriles

(Scheme 3)

8.2.1 Chemistry

1-Isonicotinoyl-4-phenylthiosemicarbazide (4) was obtained by the reaction of isonicotinic acid hydrazide with phenylisothiocyanate. The cyclization of compound (4) in the presence of 2N NaOH resulted in the formation of 4-phenyl-5-pyridin-4-yl-4[H]-1,2,4-triazole-3-thiol (5). The reaction of compound (5) with chloroacetone in presence of potassium carbonate in DMF resulted in the formation of 1-(4-phenyl-5-pyridin-4-yl-4[H]-1,2,4-triazole-3-ylthio)acetone (6). A series of title compounds (7), were then prepared by one-pot synthesis from malononitrile, aromatic aldehyde, acetone derivative (6) and ammonium acetate under microwave irradiation without solvent.

When a mixture of aromatic aldehyde a, acetone derivative b, malononitrile c, and ammonium acetate was irradiated in a microwave oven (Figure 8.6), the reactions were almost completed in 3-7 min. The reaction mixtures were then washed with a small amount of alcohol. The crude products were purified by recrystallization from 95% ethanol to afford products with good yields (70-80%). This
procedure has the advantage of short routine, good yields, convenient workup and being environmentally friendly.

\[
\begin{align*}
\text{ArCHO} & + \text{R-COCH}_3 \\
\text{NH}_4\text{OAc} & \xrightarrow{\text{MWI}} \text{d}
\end{align*}
\]

Figure 8.6

The reaction may proceed via imine (e) formed from ketone and ammonium acetate, imine (e) reacts with alkylidemalononitrile (f) (from condensation of aromatic aldehyde with malononitrile) to give (g), followed by cycloaddition, isomerization, aromatization to afford the 2-amino-3-cyanopyridine (d) (Figure 8.7).

\[
\begin{align*}
\text{b} & \quad \text{NH}_4\text{OAc} \\
\text{a} & \quad \text{CN} \quad \text{CN} \quad \text{NH}_4\text{OAc} \\
\text{c} & \quad \text{f} \\
\text{g} & \quad \text{d}
\end{align*}
\]

Figure 8.7: Mechanism of the reaction
The formation of compounds 7a-e was substantiated by spectral studies. In the $^1$H NMR spectra of compound (4), additional NH signals (controlled with D$_2$O) derived from thiosemicarbazide structure were observed at 9.65, 9.75, and 10.30 ppm, while the signal due to NH$_2$ group of hydrazide structure did not appear. Additional signals belonging to phenyl ring were observed in the aromatic region in the $^1$H NMR spectra of compound (4). When compound (4) was converted to 4-phenyl-5-pyridin-4-yl-4$H$-1,2,4-triazole-3-thiol (5) in basic media, NH peaks disappeared, while new signal due to SH group was observed at 14.36 ppm (controlled with D$_2$O) in the $^1$H NMR spectra of compound (5). It is interesting to note that thiocarbonyl compounds are present in their thione-thiol tautomeric forms in solution as indicated by their IR and $^1$H NMR spectra. These tautomeric forms are also present in dimethylsulfoxide as suggested by $^1$H NMR spectral data. When compound (5) was converted to its ketone derivative (6), the SH signal disappeared, instead, new signals that originated from CH$_3$ and CH$_2$ were observed at 4.50 and 2.78, respectively in the $^1$H NMR spectra of compound (6). In the $^1$H NMR spectrums of compound (7) displayed no signals belonging to CH$_3$ group; instead, new signals derived from NH$_2$ appeared. The $^1$H NMR spectra of compound (7) displayed additional signals due to the aromatic ring derived from aldehyde moiety at aromatic region. Moreover, the
signals derived from one OH group in compound 7c was recorded at 5.00 ppm and in compound 7e signals appeared at 2.85 attributed to N(CH$_3$)$_2$. The IR spectrum of compound (5) displayed SH stretching band 2732 cm$^{-1}$. In the $^1$H NMR spectrum of compound (5), additional signal due to SH group appeared at 13.89 ppm. The IR spectra of compounds (7a-e) showed multiple bands in the 3489-3274 cm$^{-1}$ region due to NH stretching vibrations of the amino group, bands around 1640 cm$^{-1}$ characteristic of NH bending vibrations and C≡N around 2210 cm$^{-1}$. In the mass spectra of the compounds (7a-e), molecular ion peaks were observed for all compounds at different intensity verifying the molecular weight of compound.

### 8.2.2 Biological results

4-phenyl-(5-pyridin-4-yl)-4H-1,2,4-triazole-3-thiol (5) displayed no antimicrobial activity, where as its pyridine derivative (7a-e), displayed good activities against all tested microorganisms. The result of antimicrobial screening revealed that compound 7a-e displayed better antibacterial activity compared with their antifungal activity. From the result, it was observed that the compound 7d (3-Cl) and 7c (4-OH) showed good inhibition against gram-negative organism such as E. Coli and P. aeruginosa and also against gram-positive organism such as Bacillus subtilis with MIC value 16 µg/ml but not against S. aureus having MIC value 62.5 µg/ml. The compound
7b (3-NO₂) had moderate activity against *S. aureus*, *E. coli* and *B. subtilis* with MIC value 62.5 µg/ml and against *P. aeruginosa* with MIC value 31.25 µg/ml. Among synthesized compounds, 7a and 7e showed poor activity.

The results of antifungal activity were compared with the standard Fluconazole. Almost all newly synthesized compounds showed poor activity against all types of fungal strains with MIC value between 62.5 µg/ml and 125 µg/ml.
Section-B

OXADIAZOLE

8.3 Synthesis of 2-(N-substitutedcarboxamidoalkylthio)-5-pyridine-4-yl-1,3,4-oxadiazoles, their corresponding sulfoxides and sulfones

(Scheme 4 and 5)

8.3.1 Chemistry

The synthesis of target compounds was carried out as depicted in scheme 4 and 5. Ethylisonicotinate (2), the starting material, was prepared according to the method reported in the literature, using isonicotinic acid. The isonicotinic acid hydrazide (3) was prepared by esterification of isonicotinic acid (1) followed by treatment with hydrazine hydrate in absolute ethanol. The reaction of hydrazide (3) with carbon disulfide in alkaline medium afforded, after acidic treatment, 5-(pyridin-4-yl)-1,3,4-oxadiazole-2-thiol (4). A series of title compounds (8 and 13), were then synthesized by the reaction of potassium salt of 5-(pyridin-4-yl)-1,3,4-oxadiazole-2-thiol (4) with N-substituted chloroacetamide (7) and N-substituted β-chloropropionamide (12) under Schotten-Baumann reaction condition using conventional as well as microwave irradiation method. As indicated by TLC analysis, a maximum of 5 minute of heating suffices to produce nearly complete conversion using microwave irradiation technique. However, in absence of microwave heating, poor
conversion to oxadiazole derivatives were realized when the reactions were stirred at 60-80°C for 36 h. The products obtained by conventional and microwave irradiation methods were found to be identical by their melting point, mixed melting point, elemental analysis and spectral data. The structure of various synthesized compounds was assigned on the basis of different chromatographic and spectral studies.

The formation of isonicotinic acid hydrazide (3) from ethylisonicotinate (2) was confirmed by its IR and 1H NMR. IR spectrum of (3) showed absorption band at 3300, 3110, 1680, 1550 cm⁻¹ due to NH₂, -NH, C=O and C=C groups, respectively while 1H NMR showed sharp two doublets at δ7.7 and δ8.7 indicating the presence of four aromatic protons. The cyclization of (3) to 5-(pyridin-4-yl)-1,3,4-oxadiazole-2-thiol (4) was confirmed by its IR spectrum which showed absorption bands at 1560, 1370, and 1460 due to C=N, C=S, and C-NH, respectively. In 1H NMR spectral data, all protons were seen according to the expected chemical shift and integral values. We have observed that extensive thiol-thione tautomerism exists in compound (4). In the 1H NMR the signal of the SH protons were recorded, although they were very weak and also the ready synthesis of (8 and 13) from (4) confirmed the tautomerism. It has been reported that the crystal structure of (4) correspond to the thione form, but the
reaction conditions for the synthesis of (8 and 13) proved that it can be in the thiol form too.

The –SH proton is acidic enough and some substitution reaction could be achieved on this group in the presence of a base. In the 1H NMR spectrum of compound (8), additional signals derived from –CH₂, NH and aromatic protons were observed at 2.3-4.6, 8.9-10.6 and 6.6-7.9 ppm respectively while in the 1H NMR spectrum of compound (13), the signals derived from -COCH₂, -SCH₂, NH and aromatic protons were observed at 2.3-2.9, 3.5-3.9, 9.2-9.8 and 6.8-7.8 ppm respectively. In IR spectra of compound (8 and 13) showed a peak at 3228 to 3369, 3066 to 3172, 1612 to 1681, 1554 to 1607, due to NH, Ar-CH, CO, C=N, respectively.

The synthesis of corresponding sulfoxides (9 from 8 and 14 from 13) and sulfones (10 from 8 and 15 from 13) of various derivatives of compound (8 and 13) were carried out by the reaction of compound (8) with two equivalent of oxone® in presence of one equivalent of anhydrous aluminium chloride and four equivalent of oxone® and two equivalent of anhydrous aluminium chloride, respectively. IR spectrum of the compound (9 and 14) exhibited NH stretching at 3237-3392 cm⁻¹ (absent in 9k, 9l and 14k, 14l) and SO band in the region 1130-1170 cm⁻¹. In the 1H NMR, appearance of a downfield shift at –SCH₂ and NH proton signals for sulfoxides (9) when compared with the sulfides (8) indicated that oxidation reaction
occurred. Similarly, in the $^1$H NMR spectrum of sulfoxides (14), appearance of a downfield shift at -SCH$_2$, -COCH$_2$ and NH proton signals, when compared with the sulfides (13), indicated that oxidation reaction occurred. IR spectrum of the compound (10 and 15) exhibited NH stretching at 3243-3398 cm$^{-1}$ (absent in 9k, 9l and 14k, 14l) and two series of SO$_2$ stretching bands, one between 1311 and 1360 cm$^{-1}$ and the other one between 1125 and 1170 cm$^{-1}$ were seen. In the $^1$H NMR, appearance of a downfield shift at –SCH$_2$ and NH proton signals for sulfone compounds (10) when compared with the sulfides (8) and sulfoxides (9) and similarly, appearance of a downfield shift at -SCH$_2$, -COCH$_2$ and NH proton signals for sulfone compounds (15) when compared with the sulfides (13) and sulfoxides (14) indicated that oxidation reaction occurred. But it was hard to say that isolated product has either one or two oxygen in its molecule.

Additional evidence was provided by elemental analysis and mass spectra of the compounds. In the mass spectra of the compounds (9 and 14) and (10 and 15), molecular ion peaks were observed for all compounds at different intensity verifying the molecular weight of a sulfoxide and sulfone.

**8.3.2 Biological results**

*Antimicrobial activity*

The synthesized compounds were tested for their in vitro antimicrobial activity against the gram-positive bacteria.
Staphylococcus aureus, Bacillus subtilus, the gram-negative bacteria Escherichia coli, Pseudomonas aeruginosa and fungi Candida albicans and Aspergillus niger.

The results revealed that in general, the inhibitory activity against the gram-positive bacteria was higher than that of the gram-negative bacteria species. Compounds 14k, 14l and 15k, 15l showed potent activity against gram-positive bacteria with MIC value 4 µg/ml and moderate to high activity against gram-negative bacteria with MIC value 8 µg/ml. Compounds 9k, 9l, 10k, 10l, 13k and 13l exhibited moderate activity against the both gram-positive bacteria species with MIC value 8 µg/ml and average activity against gram-negative bacteria species with MIC value 16 µg/ml. While all the tested compounds showed weak (8-10 and 13-15) to moderate inhibitory effects towards the selected pathogenic fungi.

In order to probe structural requirements for optimal antimicrobial activity in this series, the substituents on the phenyl ring, incorporation of side chain nitrogen into cyclic ring and oxidation of sulfur to sulfoxide and sulfone have been examined. In regard to structure the most important variable affecting the activity was the oxidation of sulfur to sulfoxide and sulfone. In each case, the sulfoxides and sulfones proved to be more potent than the corresponding sulfides. Moreover, little distinction and no discernable trends were observed in comparing the sulfoxides with the
corresponding sulfones and both exhibit the said activities to the same extent. In the sulfide series, compounds 8k and 8l, in which side chain nitrogen was incorporated into pyrrolidine and morpholine ring, respectively, were more active than other compounds in the series. The same trend was observed in case of sulfoxides and sulfones. Among the substituents on the phenyl ring, 4-chloro has shown greater influence on biological activity in each series.

8.4 Synthesis of 2-(N-benzylcarboxamidoalkylthio)-5-aryl-1,3,4-oxadiazoles, and their corresponding sulfoxides
(Scheme 6)

8.4.1 Chemistry

The synthesis of target compounds was carried out as depicted in Scheme 6. Aromatic carboxylic ester (2), the starting material, was prepared according to the method reported in the literature, using respective aromatic carboxylic acid. The acid hydrazide (3) was prepared by esterification of (1) followed by treatment with hydrazine hydrate in absolute ethanol. The reaction of hydrazide (3) with carbon disulfide in alkaline medium afforded, after acidic treatment, 5-aryl-1,3,4-oxadiazole-2-thiol (4).
The N-benzylchloroacetamide and N-benzyl-β-chloropropionamide (7) were prepared by acetylated benzylamine (5) using chloroacetyl chloride and β-chloropropionyl chloride (6), respectively. A series of title compounds (8) were then synthesized by the reaction of potassium salt of 5-aryl-1,3,4-oxadiazole-2-thiol (4) with N-benzyl-chloroacetamide and N-benzyl β-chloropropionamide (7) by microwave irradiation method. The reaction of these sulfides (8) with two equivalent of oxidant, Oxone® gave selectively the corresponding sulfoxides (9) in high yields. The structure of various synthesized compounds was assigned on the basis of different chromatographic and spectral studies.

The formation of appropriate hydrazide (3) from respective aromatic ester (2) was confirmed by its IR and 1H NMR. IR spectrum of (3) showed absorption band at 3300, 3110, 1675, 1550 cm⁻¹ due to NH₂, -NH, C=O and C=C groups, respectively while ¹H NMR showed multiplet at δ7.0 to δ7.8 indicating the presence of aromatic protons. The cyclization of (3) to 5-aryl-1,3,4-oxadiazole-2-thiol (4) was confirmed by its IR spectrum which showed absorption bands at 1560, 1370, and 1460 due to C=N, C=S, and C-NH, respectively. In ¹H NMR spectral data, all protons were seen according to the expected chemical shift and integral values. We have observed that extensive thiol-thione tautomerism exist in compound (4).
In the $^1$H NMR spectrum of compound (8), additional signals derived from two –CH$_2$ (in case of acetamide), three –CH$_2$ (in case of propionamide), NH and aromatic protons were observed at 4.0 to 4.5, 2.63 to 4.52, 9.3-10.3 and 5.96-8.3 ppm respectively. In IR spectra of compound (8) showed a peak at 3280 to 3291, 3062 to 3173, 1639 to 1653, 1533 to 1562, due to NH, Ar-CH, CO, C=N, respectively.

IR spectrum of compound (9) exhibited NH stretching at 3279-3289 cm$^{-1}$ and SO band in the region 1060-1150 cm$^{-1}$. In the $^1$H NMR appearance of downfield shift at –SCH$_2$ and NH proton signals for sulfoxides (9) when compared with the sulfide (8) indicate that oxidation reaction occurred. But it was hard to say that isolated product has either one or two oxygen in its molecule. Additional evidence was provided by mass spectra of the compounds. In the mass spectra of the compounds (9) molecular ion peaks were observed for all compounds at different intensity verifying the molecular weight of sulfoxide compound.

8.4.2 Biological results

Anticonvulsant activity

The existence of a hydrophobic unit, an electron donor group and hydrogen bonding domain was essential for anticonvulsant activity as depicted by the models and also evidenced by active drug Phenytoin and Hibicon. Anticonvulsant data of MES and PTZ screening revealed that compounds 8a, 8c, 8d and 9d had shown
significant activity (p<0.01) compared to standard drug Phenytoin and control group, respectively. Compound 9h was found to be significant but with p<0.05 and compounds 8e, 8f, 9f and 9g were found to be non significant.

A study of structure–activity relationship revealed that compounds (8a), (8c), (8d) and (9d) exhibited their ability to diminish tonic-extensor seizures. The extensor phase time was remarkably reduced for these compounds.

From the results obtained, following SAR can be drawn:

- In both sulfide and sulfoxide series, compounds 8d and 9d having 2-furyl substituent at 5th position of oxadiazole ring had shown significant activity compared to other substituents.

- In general, all sulfides were found more active than their corresponding sulfoxides indicated that oxidation of sulfur to sulfoxide caused decrease in activity such as compound 9d (sulfoxide) was found less active than 8d (sulfide).

- Activity decreases by increasing the carbon chain by one CH₂ group since compounds 8e, 8f, 8g and 8h were found to be less active than their corresponding lower alkyl analogs.

8.4.3 In silico docking analysis of synthesized compound 8d

Based on the results of anti-convulsant activity and to clarify the mechanism of synthesized compounds, the most active compound
was subjected to molecular docking Using Molecular Operating Environment 2009 (MOE).

**Experimental**

**SAR analysis**

SAR analysis was carried out by Field Align software Package. Field analysis aligns molecules based on their molecular fields, not on their structure. The interaction between a ligand and a protein involves electrostatic fields and surface properties (e.g. hydrogen bonding, hydrophobic surfaces and so on). To carry out SAR analysis we have calculated similarity score for scaffold \((N\text{-}benzyl\text{-}2\text{-}\{5\text{-}(\text{furan}2\text{-}yI}\text{-}\text{I},3,4\text{-}\text{oxadiazol}2\text{-}yI}\text{I}\text{sulfanyl}\text{acetamide})\) molecule and Hibicon as reference molecule. This study revealed that compound 8d is about 78% similar with Hibicon, in terms of various fields like electrostatic, steric and hydrophobic (**Figure 8.8**).

**Figure 8.8**: Field analysis of Hibicon. **Blue**: Negative field points, **Red**: Positive field points, **Yellow**: van der Waals surface field points, **Gold/Orange**: Hydrophobic field points

**EXPERIMENTAL PROTOCOLS**
Computer-assisted simulated docking experiments were carried out in human GABA crystal structure (PDB ID: 3ip9).\textsuperscript{9-10} Docking simulation study of \( \text{N-benzyl-2-}\left[5-(\text{furan-2-yl})-1,3,4-\text{oxadiazol-2-yl}\right]\text{sulfanyl}\text{acetamide} \) was carried out using Molecular Operating Environment (MOE) 2009 as depicted below.

- Enzyme structure was checked for missing atoms, bonds and contacts. Ramchandran plot was plotted to check the health of protein.
- Hydrogen atoms were added to enzyme structure. Bound ligands were manually deleted from the enzyme.
- The ligand molecules were constructed using ACD Chem Sketch 11.0 and optimized structure was used for docking.
- The active site was generated and ligands were docked within the GABA receptor active site.
- The lowest energy conformation was selected and subjected to an energy minimization.

**MODELING STUDIES**

Molecular modeling and conformational alignment studies of the \( \text{N-benzyl-2-}\left[5-(\text{furan-2-yl})-1,3,4-\text{oxadiazol-2-yl}\right]\text{sulfanyl}\) acetamide were performed in order to rationalize the obtained biological results (Figure 8.9).
Figure 8.9: (a) Energy minimized 3D conformation and (b) Molecular surface areas of N-benzyl-2-[(5-(furan-2-yl)-1,3,4-oxadiazol-2-yl)sulfanyl]acetamide. Red = mild polar, blue = H-bonding and white = hydrophobic region.

MOLECULAR DOCKING

Based on the hypothesis\textsuperscript{11} that similar molecules tend to make similar interactions with the protein, bind to a common active site in the protein and the results obtained in SAR and field analysis, the
scaffold \((N\text{-}benzyl\text{-}2\text{-}([5\text{-}(\text{furan}\text{-}2\text{-}yl]\text{-}1,3,4\text{-oxadiazol}\text{-}2\text{-}yl]\text{sulfanyl})acetamide)\) was subjected to the molecular docking.

Before molecular docking the 3D structure of ligand was optimized. The PDB ID: 3ip9 was subjected to energy and residue optimisation. The health of protein was checked by plotting Ramchandran plot (**Figure 8.10**).

![Ramchandran Plot for pdb 3ip9 after energy and residue optimization.](image)

**Figure 8.10**: (a) Docking pose of \(N\text{-}benzyl\text{-}2\text{-}([5\text{-}(\text{furan}\text{-}2\text{-}yl}\text{-}1,3,4\text{-oxadiazol}\text{-}2\text{-}yl]\text{sulfanyl})acetamide in the active site of GABA receptor (b) Ramchandran Plot for pdb 3ip9 after energy and residue optimization.

Docking analysis of \(N\text{-}benzyl\text{-}2\text{-}([5\text{-}(\text{furan}\text{-}2\text{-}yl}\text{-}1,3,4\text{-oxadiazol}\text{-}2\text{-}yl]\text{sulfanyl})acetamide into the active site of GABA receptor revealed that oxadiazole ring of the scaffold showed strong hydrogen bond interaction with serine (79) residue with the percent of hydrogen bonding was 37% and interatomic distance was maintained at 1.5 \(\text{A}^0\)

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while aromatic ring near amide linkage signify arene-arene interaction with phenylalanine (77) residue (as shown in Figure 8.11). Arene–arene interaction was characterized as hydrophobic interaction between scaffold and GABA receptor. Aromatic nucleus constitutes an important part in scaffold, accountable for the hydrophobicity of scaffold. In Hibicon, aromatic ring maintain the hydrophobicity as well as elicit the hydrophobic interaction with GABA receptor (as shown in Figure 8.12) through arene-arene contact. So presence of aromatic ring is vital for the drug to act as potent anticonvulsant agent.

Further residues Ala (100), Asp (226), Gly (227), Tyr (150), Leu (202), Thr (148) which approaches closely to the ligand but do not have any qualifying strong interactions (i.e. hydrogen bonds). It may be classified as non-bonded residues that have a significant effect on the orientation and binding of the ligand, but which may be spread out over a number of pairwise contacts, each of which is relatively weak.
**Figure 8.11:** Showing interaction of \(N\)-benzyl-2-\{[5-(furan-2-yl)-1,3,4-oxadiazol-2-yl]sulfanyl\}acetamide with the active site amino acid residue of GABA receptor. Nitrogen atom of oxadiazole nucleus acts as strong H-Bond acceptor (indicated by blue lines toward nitrogen atoms).

![Figure 8.11](image)

**Figure 8.12:** (a) showing interaction of Hibicon with the active site amino acid residue of GABA receptor. (b) Receptor based electrostatics regions in the active site of GABA receptor. Blue = donor, Red = acceptor and White = hydrophobic regions.

In addition, aromatic ring in \(N\)-benzyl-2-\{[5-(furan-2-yl)-1,3,4-oxadiazol-2-yl]sulfanyl\}acetamide have shown accountable hydrophobic interaction with receptor (as shown in **Figure 8.13**). In order to get useful results that may be valuable in future drug designing, we have carried out receptor based electrostatic analysis also.

![Figure 8.12](image)
Interestingly, aromatic nucleus which is adjacent to the amide functionality, stoutly involved in hydrophobic interaction with the receptor [as shown by white region around aromatic ring Figure 8.13(b)]. Hence presence of amide moiety and hydrophobic group adjacent to each other is favourable for activity and further substitution of aromatic nucleus with any bulky substituents may enhance hydrophobic interaction with the receptor.

Further, oxadiazole nucleus has shown strong electrostatic interaction with receptor surface as nitrogen of oxadiazole functionality acts as hydrogen bond acceptor (as shown by red region around oxadiazole nitrogen’s in Figure 8.13).

Substitution of oxadiazole nitrogen with any electronegative functional group enhances the electrostatic interaction with receptor which in turn beneficial for anticonvulsant activity. Presence of furan ring at 5th position of oxadiazole enhances the hydrophobicity of

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Figure 8.13: (a) Structure of N-benzyl-2-[(5-(furan-2-yl)-1,3,4-oxadiazol-2-yl)sulfanyl]acetamide (b) Receptor based electrostatics regions in the active site of GABA receptor. Blue = donor, Red = acceptor and white = hydrophobic regions.
the scaffold and further substitution on furan ring with any bulky group may increase the affinity of scaffold toward GABA receptor.

FIELD ANALYSIS

Moreover, N-benzyl-2-\{5-(furan-2-yl)-1, 3, 4-oxadiazol-2-yl\}sulfanyl acetamide have shown remarkable field similarity with the Hibicon (Figure 8.14).

![Field analysis view of N-benzyl-2-\{5-(furan-2-yl)-1,3,4-oxadiazol-2-yl\}sulfanylacetamide and Hibicon showing field similarity up to 0.78 (78%). Blue: Negative field points, Red: Positive field points, Yellow: van der Waals surface field points, Gold/Orange: Hydrophobic field points. Results of field analysis revealed the electronegative nature of two nitrogen atoms of oxadiazole nucleus. Further aromatic ring adjacent to the amide linkage and furan ring near oxadiazole nucleus have shown characteristic hydrophobic field points similar to the receptor based electrostatic analysis (as shown in Figure 8.14).

Presence of steric field is important for the drug-receptor interaction. Comparative field analysis of N-benzyl-2-\{5-(furan-2-yl)-1,3,4-oxadiazol-2-yl\}sulfanylacetamide and Hibicon have shown
characteristic and interesting Vanderwaals field point around the various part of the scaffold and thus steric factor may be significantly involved in drug receptor interaction.  

Receptor based pharmacophore modeling  

Receptor based pharmacophore modeling was carried out by using MOE 2009 programme. The purpose of the pharmacophore modeling is to effect 3D searches of conformation databases using molecule annotations related to ligand-receptor binding (like, H-bond donor, acceptor, hydrophobe etc.). A pharmacophore is a set of structural features in a ligand that are related to the ligand’s recognition at a receptor site and its biological activity.  

Pharmacophoric structural features are represented by labeled points in space about a confirmation of the ligand. Each ligand conformation is assigned a set of annotation points, which is a set of structural features that may contribute to the ligand’s pharmacophore.  

Pharmacophore modelling of \(N\)-benzyl-2-\{\(\text{[5-(furan-2-yl)-1,3,4-oxadiazol-2-yl]-sulfanyl}\)}acetamide have shown three characteristic hydrophobic region (as shown by green contours in Figure 8.15) namely aromatic ring, sulfur bridge and furan substituent. Aromatic ring facing the hydrophobic surface of the receptor (Figure 8.15) signifies that substitution of aromatic ring is beneficial for
anticonvulsant activity. So further substitution of hydrogen bond acceptor at ortho position of aromatic ring enhances the electrostatic interaction with receptor, whereas, para position is directing towards hydrophobic surface of GABA receptor and there may be requirement of any bulky group that impart hydrophobicity to the scaffold.

Figure 8.15: Showing various pharmacophoric feature of N-benzyl-2-[[5-(furan-2-yl)-1, 3, 4-oxadiazol-2-yl] sulfanyl] acetamide in GABA receptor pocket. (Receptor surface reveals hydrogen bonding region by blue colour, hydrophobic region by white colour and mild polar region by red colour. Pharmacophoric features were represented by coloured contours: green colour indicate hydrophobic region of scaffold, sky blue shows hydrogen bond acceptors and pink colour represent hydrogen bond donors.)
Furan ring showing characteristic green contour revealed the hydrophobic nature of furan and align itself towards hydrophobic region of GABA receptor (as shown in Figure 8.15). So the presence of furan ring enhances the hydrophobicity of scaffold and beneficial for anticonvulsant activity. The carbon chain connecting the aromatic ring to the oxadiazole nucleus impart flexibility to the structure of scaffold and significantly involved in flexible alignment of structure into the GABA receptor pocket and in the same way, carbon chain connecting the aromatic ring and amide functionality impart excellent flexibility to the structure to acquire desire conformation into the GABA receptor pocket (as shown in Figure 8.15).

**Figure 8.16:** Showing various pharmacophoric features of N-benzyl-2-[[5-(furan-2-yl)-1, 3, 4-oxadiazol-2-yl] sulfanyl] acetamide. (Don denotes H-bond donor, Acc denotes H-bond acceptor, Hyd denotes a
hydrophobic center, and Aro denotes an aromatic center projected annotation points).

Sulfur bridge connecting amide functionality and oxadiazole nucleus showing green contour revealed the hydrophobic nature of sulfur in scaffold (Figure 8.16). Sulfur atom align itself towards hydrophobic region of GABA receptor indicates that presence of sulfur bridge is important for anticonvulsant activity and may impart flexibility to the scaffold to acquire desired conformation within the GABA receptor active site.

Oxadiazole constitute central part of the molecule in which two nitrogen atoms are strong electronegative centres and acts as hydrogen bond acceptor (as shown in Figure 8.16) in drug receptor interactions. Oxadiazole nucleus aligns itself towards hydrogen bonding region of GABA receptor, facing the two nitrogen towards H-bonding surface of GABA, acting as hydrogen bond acceptor. It is important for electrostatic interaction with GABA receptor, while oxygen atom of oxadiazole have not shown any involvement in drug receptor interaction. It is hypothesized that, there may be occurrence of ring chain tautomerism in oxadiazole nucleus which may improve the electronegativity of oxadiazole nitrogen and boost affinity toward GABA receptor.

Amide linkage connecting aromatic ring and sulfur bridge shows two important pharmacophoric features i.e. oxygen atom acts as hydrogen bond acceptor and nitrogen atom acts as hydrogen bond.
donor. There may be possibility of amide-amidic tautomerism in amide linkage leading to the formation of hydroxy group in vivo which may enhance electrostatic interaction with GABA receptor and hydroxy group formed by tautomerism may prove to be potential hydrogen bond acceptor in drug receptor interaction.
8.5 Synthesis of 2-amino-6-[[5-(2-chlorophenyl)-1,3,4-oxadiazole-2-ylthio]methyl]-4-arylnicotinonitriles

(Scheme 7)

8.5.1 Chemistry

The formation of compounds 6a-e was characterized by spectral studies. All spectral data were in accordance with the assumed structures. Ethyl-2-chlorobenzoate (2), the starting material, was prepared according to the method reported in the literature, using 2-chlorobenzoic acid. The acid hydrazide (3) was prepared by esterification of 2-chlorobenzoic acid (1) followed by treatment with hydrazine hydrate in absolute ethanol. The reaction of hydrazide 3 with carbon disulfide in an alkaline medium afforded 5-(2-chlorophenyl)-2-mercapto-1,3,4-oxadiazole (4). The reaction of compound (4) with chloroacetone in presence of potassium carbonate in DMF resulted in the formation of 1-[[5-(2-chlorophenyl)-1,3,4-oxadiazole-2-ylthio]acetone (5). A series of title compounds (6), were then prepared by one-pot synthesis from malononitrile, aromatic aldehyde, ketone derivative and ammonium acetate under microwave irradiation without solvent.

The formation of 2-chlorobenzoic acid hydrazide (3) was confirmed by its IR and $^1$H NMR spectrum. IR spectrum of (3) showed absorption band at 3310, 3140, 1675, 1555 cm$^{-1}$ due to NH$_2$, -NH, C=O and C=C groups, respectively while $^1$H NMR showed multiplet at
7.7 to 8.2 integrating for four aromatic protons and singlet signal derived from hydrazide (-NH-NH₂) structure appeared at 4.20 ppm.

The cyclization of (3) to 5-(2-chlorophenyl)-1,3,4-oxadiazole-2-thiol (4) was confirmed by its IR spectrum which showed absorption bands at 1167, 1250, 1610 cm⁻¹ due to C=O-C, C=S and C=N, respectively while the signals belonging to –NHNH₂ disappeared indicating cyclization of hydrazide to oxadiazole.

In ¹H NMR spectra of compound (5), new signals that originated from –CH₂ and –CH₃ were observed at 4.50 and 2.78 respectively. IR spectra exhibited characteristics peak at 1710 cm⁻¹ due to carbonyl function derived from ketone structure but it was disappeared in the IR spectra of compound (6). Moreover, the IR spectra of compound 6a-e showed multiple bands in the 3400-3200 cm⁻¹ region due to NH stretching vibration of the amino group, bands around 1640 cm⁻¹ characteristic of NH bending vibrations and CN band around 2210 cm⁻¹.

In the ¹H NMR spectrum of compound (6) displayed no signals belonging to CH₃ group; instead, new signals derived from –NH₂ appeared at 4.0 ppm and additional signals due to the aromatic ring derived from aldehyde moiety at aromatic region. Moreover, the signals derived from one OH group in compound 6c was recorded at 5.00 ppm and in compound 6e signal appeared at 2.85 attributed to
N(CH$_3$)$_2$. The mass spectrum of 6a-e showed a molecular ion peak which corresponding to its molecular weight. Other peaks appearing were in accordance with the fragmentation pattern. Elemental analysis supported the structure of various synthesized compounds.

### 8.5.2 Biological results

The synthesized compounds were tested for their in vitro antimicrobial activity against the gram-positive bacteria *Staphylococcus aureus*, *Bacillus subtilis*, the gram-negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa* and fungi *Candida albicans* and *Aspergillus niger*. The results revealed that in general, the inhibitory activity against the bacterial species was higher than that of the fungal species. Compounds 6c showed good activity against *B. subtilis*, *P. aeruginosa* and *E.coli* and moderate activity against *S. aureus*. While all the tested compounds showed weak to moderate inhibitory effects towards the selected pathogenic fungi.
8.6 References


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